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Automated one-step supercritical fluid extraction and clean-up system for the analysis of pesticide residues in fatty matrices

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Abstract

An automated supercritical fluid extraction and in-line clean-up system has been developed for organochlorine and organophosphate pesticide residues contained in fats. This procedure utilizes supercritical carbon dioxide modified with 3% acetonitrile at 27.58 MPa and 60°C to extract and separate the pesticide residues from the fat on a C_1 bonded phase preparative column at 95°C. The automated C_1 system recovers 86 of 117 nonpolar to moderately polar organochlorine and organophosphate pesticides from fats. Ten of the 31 pesticides not recovered through the system are not recovered through the conventional clean-up sorbent, Florisil. Pesticide residues can be separated from 0.68 g of butter fat and 0.67 g corn oil, resulting in 2.9 mg of butterfat and 2.1 mg corn oil residue co-eluting into the pesticide fraction. Also, this integrated method can extract and clean-up a 5 g sample of fatty foods containing <18% fat and 70% moisture. The automated C_1 system is reproducible and the amount of co-extracted sample residue in the pesticide fraction yields results comparable to the current methodology, which uses organic solvent extraction and gel permeation chromatography, along with a final Florisil column clean-up step. This automated C_1 system simplifies the extraction and clean-up step while reducing solvent usage and hazardous waste.

Keywords: Automation; Sample handling; Food analysis; Fats; Pesticides; Organochlorine compounds; Organophosphate compounds

1. Introduction

The analytical methodology for determining organochlorine and organophosphate pesticide residues in fats and oils uses different procedures as reviewed by Liem et al. [1]. They all require organic solvent extraction of the pesticide residues and fat from the matrix followed by subsequent clean-up of the extract to achieve a suitable sample for gas chromatographic determination. Such pesticide residues are separated from the lipids using techniques, which include liquid–liquid partitioning, gel permeation chromatography (GPC), or sweep co-distillation. These pesticide residues are further cleaned up by adsorption column chromatography using Florisil, alumina, silica or solid-phase extraction cartridges before their determination by gas chromatography (GC). Pesticide residues are usually detected with selective detection methods which include electrolytic conductivity detection (ELCD) in the halogen mode and electron-capture detection (ECD) for organochlorine pesticides or flame photometric

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detection (FPD) in the phosphorus mode for organophosphate pesticides.

Most laboratories use combinations of the above procedures to analyze fatty foods for pesticide residues and several of these procedures are included in the proven methodologies contained in the Pesticide Analytical Manual Volume I (PAM I) [2]. Several of the liquid-based extraction and clean-up procedures are labor intensive, consume large amounts of solvents and reagents and generate considerable quantities of hazardous waste.

Alternative procedures which include supercritical fluid extraction (SFE) with CO_2 [3–6] or accelerated extraction with heated fluids [5,7–9] have shown that they are equivalent to the liquid-based extraction procedures for extracting pesticide residues and lipids from fatty matrices. These alternative extraction procedures, which can be automated, reduce solvent usage, hazardous waste, and simplify the extraction, however the pesticide residues still need to be separated from the coextracted lipid matter.

Alternative supercritical fluid clean-up procedures have shown that pesticide residues can be cleaned up from fats by adding 0.2 g of extracted fat to alumina contained in an extraction vessel and extracting with supercritical CO_2 [10]. Also, pesticide residues can be cleaned up from extracted fat by using supercritical CO_2 with an alumina column and modified supercritical CO_2 with a silica column [11] after being loaded into a loop injector.

In this study, an automated one-step SFE and in-line clean-up system has been developed for organochlorine and organophosphate pesticide residues contained in fats and fat samples. This procedure utilizes supercritical carbon dioxide modified with 3% acetonitrile at 27.58 MPa and 60°C to extract and separate the pesticide residues from the fat on a C_1 bonded phase, preparative column at 95°C [12].

2. Experimental

2.1. Reagents and materials

Commercial grade liquid Bone Dry carbon dioxide (Linweld Gas Supply, Kansas City, MO, USA) was used in each extraction. Samples were cleaned up on two C_1 silica based preparative 250 mm×10 mm columns containing 5 μm spherical silica loaded with C_1 packing with >98% end capping [No. C1-P; Advanced Separations Technologies (ASTEC), Whippany, NJ, USA]. USP grade 95% ethanol and pesticide grade methylene chloride, *n*-hexane, diethyl ether, light petroleum (b.p. 30–60°C), acetonitrile, acetone, isopropanol and isooctane were used in this procedure. Pyrex fiber glass wool (No. 3950; Corning, Corning, NY, USA) was used to dry wash the glass rod, beaker, and powder funnel after packing the sample in the extraction cell. A Kimax, 60 mm diameter top×13 mm O.D. stem powder funnel (No. 10-346-5A; Fisher Scientific, Pittsburgh, PA, USA) was used in packing the extraction vessels. A 10-ml and 25-ml graduated cylinder (Nos. 3022-10, 3022-25; Corning). A 180 mm×6.4 mm glass rod with ends fire polished (No. 743070; Corning) was used in mixing the sample. A 50-ml beaker (No. 1000-50; Corning) was used in mixing Chem Tube Hydromatrix material (No. 0019-8004; Varian, Harbor City, CA, USA) a dispersing agent with the sample. Pesticide residue grade Florisil, 60–100 mesh (Floridin, Berkeley Springs, WV, USA) was prepared as described [13] and used for further clean-up. Acetone–isopropanol (70:30, v/v) was used as a rinsing solvent for the C_1 columns. All pesticide standard mixtures were prepared from 1 mg/ml stock solutions dissolved in acetone–isooctane (10:90, v/v). All standards were obtained from the US Environmental Protection Agency (EPA), Pesticide and Industrial Chemicals Repository, Research Triangle Park, NC, USA.

2.2. Apparatus

2.2.1. Extraction and clean-up system

The automated C_1 system in Fig. 1 consists of CO_2 fed through the clean-up trap to a Isco Model 260 D CO_2 syringe pump (Isco, Lincoln, NE, USA) with its pump cylinder cooled (to 0°C) with a RTE-110 heater/cooler (Neslab Instruments, Portsmouth, NH, USA). The Model 260 D pump is connected through a tee to a Isco Model 100 DM modifier syringe pump containing acetonitrile and a Isco Controller. This tee is connected to a Isco SFX 3560 auto extractor operated with system software modified by Isco. The outlet line from the SFX 3560

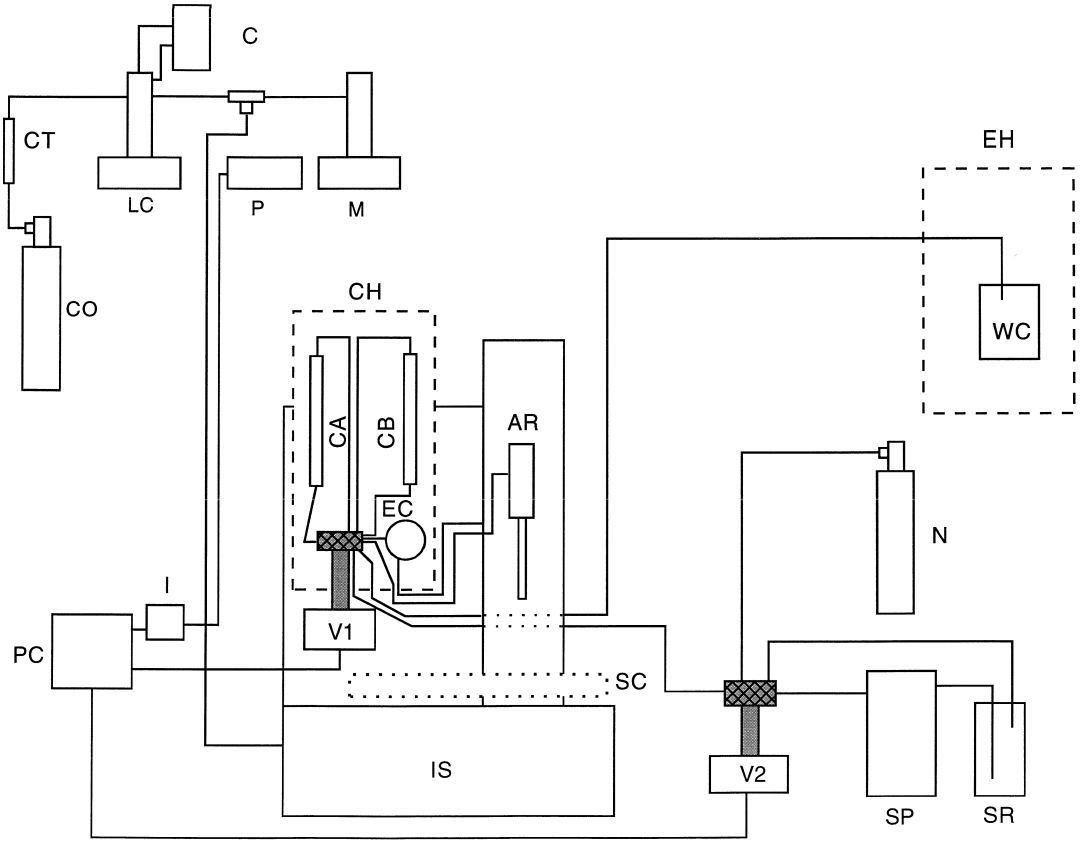

Fig. 1. Schematic diagram of the automated C_1 clean-up system used for the analysis of pesticide residues in fatty matrices. AR=Auto restrictor; C=cooler; CA=column A; CB=column B; CH=column heater; CO=commercial grade CO_2 (bone dry); CT=CO_2 clean-up trap; EC=equilibration coil; EH=exhaust hood; I=inverter (logic interface); IS=auto-extractor; LC=liquid CO_2 pump; M=modifier pump; N=nitrogen at 1.379 MPa; P=pump controller; PC=programmable controller; SC=sample carousel; SP=solvent pump for rinsing columns; SR=solvent reservoir; V1=valve 1 (eight-port) for switching between column A and B; V2=valve 2 (10-port) for switching between the rinsing solvent and nitrogen; WC=waste container.

extraction chamber was disconnected from the variable restrictor and connected to a 50 cm equilibration coil (Alltech, Deerfield, IL, USA) which is contained in the heated zone of a Timberline TL-430 column heater, controlled by a Timberline TL-50 Digital Temperature Controller (Alltech). The outlet of the equilibration coil is connected to port 1 of valve 1 [an eight-port 0.1588 cm valve with electric actuator (Valco, Houston, TX, USA)] as shown in Fig. 2. One of two C_1 silica-based columns is connected to ports 2 and 6 (column A) and the other column is connected to ports 8 and 4 (column B). Also, the C_1 columns and valve 1 are contained in the heated zone at 95°C. Port 5 of valve 1 is connected to the variable restrictor of the SFX 3560 extractor and the restrictor effluent is trapped in hexane.

Port 7 of valve 1 is connected to port 6 of valve 2 [10-port 0.1588 cm valve with electric actuator (Valco)] as noted in Fig. 2 and port 3 of valve 1 is connected to a waste container contained in a hood. Port 7 of valve 2 is connected to a liquid dispensing TSP minipump (Alltech) and port 8 of valve 2 is connected to the solvent reservoir of the liquid dispensing pump. Each column is rinsed with 36 ml of rinsing solvent (2 ml/min) after each clean-up step. Also, the column used with carousel sample number 1 has to have been rinsed before the next automated run. Port 4 of valve 2 is plugged and port

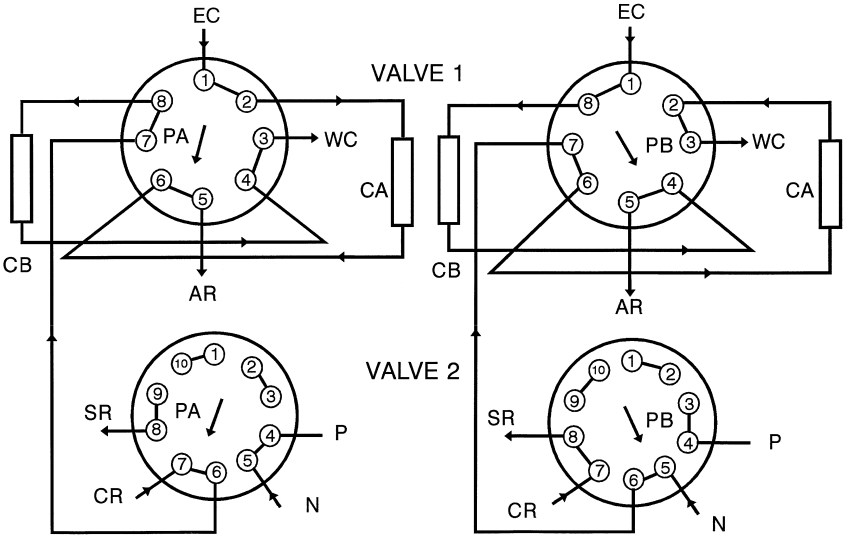

Fig. 2. Schematic diagram of peripheral switching valves 1 and 2. AR=Output from column to auto restrictor; CA=column A; CB=column B; CR=column rinsing solvent; EC=output from extraction chamber to column; N=nitrogen at 1.379 MPa; P=port 4 is plugged; PA=position A of valves 1 and 2; PB=position B of valves 1 and 2; SR=solvent reservoir; WC=waste container.

5 of valve 2 is connected to a 1.724 MPa regulator on a nitrogen tank (Linweld Gas Supply) which is set at 1.379 MPa. The rinsing solvent is pumped back into the reservoir while the column is purged with nitrogen. 316 stainless steel tubing (0.1588 cm O.D.×0.051 cm I.D., Alltech) was used for all connections between the valves and the SFX 3560.

The software for the SFX 3560 was modified by Isco so that port 7 of the pump controller is open on every odd sample and closed on every even sample. The SFX 3560's current software contains the control function needed for this application which can be made functional by Isco upon request. The signal from port 7 through a logic inverter Fig. 3 is connected to inputs 1 and 2 of a System IV programmable controller (Cole-Parmer, Vernon Hills, IL, USA) which switches valve 1 (eight-port valve) between positions A and B so that it alternates between each C_1 column, thus the fat retained on the column from the previous clean-up step can be rinsed off with solvent and the solvent is flushed from the column with nitrogen. Also, the programmable controller switches valve 2 (10-port valve) between positions A and B which alternates between the rinsing solvent and nitrogen.

A trap for CO_2 purification was constructed from 316 stainless steel tubing (No. 20-562-316; a reducing coupling, No. 20F4M16M; Butech, Erie, PA, USA) pressure-rated to 137.9 MPa at 54.2°C. Tubing dimensions were 60.96 cm×1.43 cm I.D.×2.54 cm O.D. machined on one end to accept a 10 μm stainless steel frit (No. 1000-.625-.125-10-A; Mott Metallurgical, Farmington, CT, USA). The trap was filled with a mixture consisting of 24 g coconut

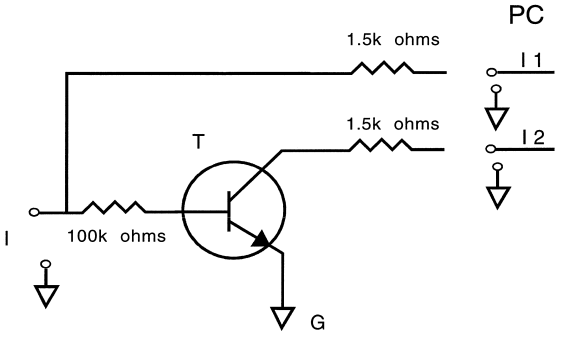

Fig. 3. Schematic diagram of logic interface inverter. I=Input from port 7 of the pump controller; I 1=input for position A of valve 1 (when the output from port 7 is ''closed'' inlet 1 is energized); I 2=input for position B of valve 1 (when the output from port 7 is ''open'' inlet 2 is energized); G=ground; PC=programmable controller inputs which are at 18 V d.c.; T=a 2N333 (NPN) transistor.

charcoal (No. 5-690-B; Fisher Scientific) and 48 g alumina C (No. 12103-99; Scientific Adsorbents, Atlanta, GA, USA) which was held in place with the 10 μm frit and a plug of glass wool. CO_2 exited through the frit end of the trap. The charcoal and alumina C were conditioned at 160°C for 18 h before being packed into the column. The clean-up trap was purged with 500 l of gaseous CO_2 before being used. This commercial grade CO_2 supplied to our laboratory after passing through the clean-up trap was adequate for trace pesticide residue analysis using GC with an electrolytic conductivity and/or a flame photometric detector.

Organochlorine pesticide residues were quantitated on a Varian 3600 system (Varian, Santa Clarita, CA, USA). This system contained a 30 m×0.53 mm DB-1, 1.5 μm film thickness, fused-silica open tubular (FSOT) column (J&W Scientific, Folsom, CA, USA) with a helium flow-rate of 25 ml/min. The column is attached to a Hall 1000 electrolytic conductivity detector operating in the halogen mode (Tremetrics, Austin, TX, USA). The injection system incorporated a direct flash vaporization inlet at 230°C. Each GC run consisted of a linear temperature program beginning at 150°C for 1 min, followed by a ramp to 250°C at 7°C/min, and finally a hold at 250°C for 10 min.

Organophosphate pesticide residues were quantitated on a Hewlett-Packard 5890 Series II system (Hewlett-Packard, Wilmington, DE, USA). This system contained a 30 m×0.53 mm DB-17, 1.0 μm film thickness, FSOT column (J&W Scientific) with a helium flow-rate of 37 ml/min. The column is attached to a flame photometric detector operating in the phosphorous mode. The injection system incorporated a direct flash vaporization inlet at 230°C. Each GC run consisted of a linear temperature program beginning at 150°C for 0 min, followed by a ramp to 230°C at 5°C/min, and finally a hold at 230°C for 15 min.

The fatty food items used in this study were obtained from the Total Diet Study [14]. Each item was cooked or prepared according to recipes used by the average American household. Large quantities of each prepared item were ground in a Robot Coupe R-10 food processor (Robot Coupe USA, Ridgeland, MS, USA) or blended in a commercial blender (Waring Products Division, New Hartford, CT, USA) into a composite. Each composite represented items purchased from three different locations within a specific region of the USA. For the purposes of these studies the USA is divided by the Food and Drug Administration into four regions, all of which are systematically sampled.

2.3. Procedure

Prepare the automated C_1 system for operation by first turning on the pump controller and pumps. Then turn on the SFX-3560 extractor and all the other peripherals except for the rinsing solvent pump. Set the parameters for the SFX-3560 as listed in Table 1 and program the programmable controller as listed in Table 2. Allow the system to stabilize to the settings. Fill all reservoirs with appropriate solvents and start the solvent rinse pump after valve 2 has switched to position B which purges the column with nitrogen.

Weigh up to 0.75 g of oil and up to 0.75 g of extracted fat, warmed until it is oil like, into a tared extraction vessel (10 ml) which has been filled with Hydromatrix material. Add 0.5 ml of water to each

Table 1
SFX 3560 method steps and parameters

Step	Operation	
1	Extraction chamber pressure (MPa)	27.58 MPa
2	Extraction chamber temperature (°C)	60
3	Restrictor temperature (°C)	100
4	Collection temperature (°C)	20
5	Restrictor flow-rate (ml/min)	1.5
6	Static extraction time (min)	5
7	Dynamic extraction time (min)	0.1
8	Static extraction time (min)	0.1
9	Set modifier volume (%)	3.0
10	Maximum extraction volume (ml)	70.0
11	Dynamic extraction time (min)	49.0
12	End program	
	Chamber wash time (s)	30
	Number of vial washes	1
	Solvent replenish 0.5 ml every (min)	1.0
	Pressurized collection	On
	Precool collection vial	On
	Post heat collection vial (degas)	Off
	Refill of pump during extraction	On
	Refill of pump before extraction	On
	Dedicated collection vial No.	0
	Solvent added before extract (ml)	9.0
	Post extraction delay time (min)	0

Table 2
Programmable controller sequence

Pump controller port 7	Input	Program	Output
Output closed	1	Turn on; delay output for 3.0 s; turn output 1 off;	Contact closure for position A valve 1 Output looped to input 3
Output open	2	Turn on; delay output for 3.0 s; turn output 2 off;	Contact closure for position B valve 1 Output looped to input 3
	3	Turn on; delay output for 18.00 min; turn output 3 off;	Contact closure for position A valve 2 Output looped to input 4
	4	Turn on; delay hold for 17.99.5 min; turn output 4 on;	Contact closure for position B valve 2

extraction vessel and install the cap. This simulates sample extraction conditions for fatty food items (<18% fat and 70% moisture).

Weigh 4–5 g of a fatty sample (<18% fat and 70% moisture) into a 50 ml beaker. Add 2–2.7 g of Hydromatrix material to the beaker and mix into a homogeneous mixture using a glass rod. Pack the resultant mixture into the extraction vessel with aid of powder funnel and glass rod. Wipe the glass rod, beaker, and powder funnel with a pledget of glass wool. Add the glass wool to extraction cell, add 0.5 ml of water to the extraction vessel if the sample contains <10% (w/w) moisture, and install cap.

Enter sample information into the SFX-3560 sample file and load the sample vessels into the auto-extractor keeping the same vessel position as it was filled (do not invert vessel). Load the appropriate collection tubes and extract the samples. The first collection tube of the run should contain 5 ml of hexane because as the internal lines of the extractor are purged, this delivers less than 9 ml of hexane to the tube. Also, using the system software can manually purge the internal lines. A large number of sample eluates can be analyzed by ELCD operating in the halogen mode or FPD operating in the phosphorous mode without further clean-up. Further clean-up by Florisil column (4 g) [2] should be used for problematic sample extracts and extracts being analyzed by ECD. Sample eluates are diluted to an appropriate volume (0.5–1.0 ml) and analyzed by GC.

3. Results and discussion

The automated C_1 system as described in Section 2.2 was based on using two C_1 columns. One column is used in the extraction and in-line clean-up step while the other column is being flushed with solvent and nitrogen thus preparing it for the next sample. This automated C_1 system was evaluated with several extracted fats, pesticide standards fortified in fat, and fat samples containing incurred pesticide residues using two C_1 columns from ASTEC which were used in the initial work [12]. These results showed that the automated C_1 system gave results similar to the initial manually operated single C_1 column system. Both systems used the following parameters: system pressure, 27.58 MPa; extraction cell temperature, 60°C; C_1 columns temperature, 95°C; static extraction, 5 min with CO_2; dynamic extraction, 0–80 ml of pump stroke volume of liquid CO_2 containing 3% (v/v) acetonitrile at a flow of 1.4 ml/min.

Also, different column rinsing solvents were evaluated on the above automated C_1 system. Mixtures of acetone and hexane, methanol, methylene chloride, and mixtures of acetone and isopropanol were tried. An acetone–isopropanol mixture (70:30, v/v) was found to be the most efficient column rinsing solution.

The automated system was further evaluated to see if other columns from different manufacturers would give results similar to the ASTEC C_1 columns. A

Hypersil SAS C_1, 120 Å, 5 μm, 250 mm×10 mm (Alltech) and a Zorbax SB-C_3, 250×4.6 mm (MAC-MOD Analytical, Chadds Ford, PA, USA) were evaluated on the automated C_1 system. Each column was evaluated with fats and pesticide standards fortified in butter fat using the above-automated C_1 conditions, but none of the columns gave results similar to the original C_1 columns from ASTEC. Each column was further evaluated with different operational parameters, but none of the columns were able to reproduce the results achieved with the C_1 columns from ASTEC. The ASTEC C_1 column, at this time, is the column of choice for this particular automated SFE and in-line clean-up system.

The automated C_1 system was evaluated with new C_1 columns from ASTEC because with use, the efficiency of the initial columns had deteriorated. The new columns for a limited number of pesticides gave results similar to the initial columns, except no recovery was recorded for endrin due to its degradation. The manufacturer indicated that the difference between the initial columns and new columns is probably caused by the base silica substrate, and unfortunately the silica substrate used in the initial columns is no longer available.

ASTEC prepared two C_1 columns from the current base silica substrate which were pre-conditioned similar to the original substrate. These columns gave results for a limited number of pesticides as recorded on the initial columns, but low recoveries for endrin (10–18%). Hence, these two C_1 columns (250 mm× 10 mm), prepared by the pre-conditioning process, were used for an in-depth evaluation of the automated C_1 system.

The effect of moisture in the extraction cell was evaluated because almost all fat samples contained more than 10% moisture which resulted in water being trapped in the collection vessel. This evaluation showed that water dissolved in the CO_2 caused pesticides to elute faster as compared to pesticides extracted with dry CO_2. Consistent elution results were achieved when 0.5 ml of water is added to all samples containing $<$10% moisture.

The elution volume of the pesticides for the automated C_1 system with the new columns was determined to be 85 ml. This 85 ml volume was changed to 70 ml based on having at least 0.5 ml of water in the extraction vessel. The automated C_1 system using a 0–70 ml elution volume of 3% acetonitrile in carbon dioxide gave results similar to the 0–85 ml elution volume without water. The automated C_1 system was further evaluated using a 0–70 ml elution volume with the method steps and parameters shown in Table 1. Steps 7 and 8 in the extraction method are used so the C_1 column can be pressurized with CO_2 without the instrument sensing it has a leak and shutting off.

Both columns were evaluated with 117 pesticide residues fortified in butter fat with 0.5 ml of water added to the extraction vessel. Table 3 shows that 31 pesticides were not recovered through the clean-up procedure and endrin gave low recoveries. Pesticides with an (*) were not recovered through the C_1 automated clean-up system. Also, the results for both columns are shown in Table 3 because all the odd numbered samples in the carousel will be cleaned up on one column and all of the even numbered samples on the other column. The results from each column are comparable within experimental error, which assures consistent results between samples.

The main difference between the initial columns and the current columns is that the initial columns allowed recovery of captan, folpet, and phorate sulfone, and gave good recoveries for endrin as opposed to the current columns. The elution differences between the initial and current columns is apparently due to the differences in the column packing substrate because all of the columns meet the manufacture's elution specifications for this particular column.

Butter fat, corn oil, soybean oil, olive oil and canola oil were cleaned up on the automated C_1 system using 0.5 ml of water in the extraction vessel. Table 4 shows that the residue left in the 0–70 ml pesticide fraction ranged from 2.8 mg (column A) to 2.9 mg (column B) for butter fat and 0.6 mg (column A) to 0.9 mg (column B) for corn oil. The residue left in the pesticide fraction from column A and B is similar to the residue left in the initial work [12].

Six samples with incurred pesticide residues were cleaned up by the automated C_1 system and the results in Table 5 are comparable to the initial Total Diet Study results. The residue left in the pesticide

Table 3
Recoveries of organochlorine and organophosphate pesticides fortified in butter fat and cleaned up through automated C_1 system

	Standard (μg)	Recovery (%) from 0–70 ml eluate		Alternate Florisil eluate[d]
		Column B	Column A	
trans-Nonachlor	0.05	96	90	1
Hexachlorobenzene	0.05	92	84	1
p,p-DDT	0.10	97	102	1
Pentachloroaniline	0.05	92	82	1
Pirimiphos methyl	0.50	88	81	3
α-BHC	0.05	90	100	1
p,p-Methoxychlor	0.30	89	88	2
Lindane	0.05	93	96	1
Heptachlor	0.05	91	90	1
p,p-DDE	0.10	115	118	1
p,p-Dicofol	0.20	102	103	1&[c]2
Diazinon	0.40	84	78	3
Dieldrin	0.10	108	107	2
Parathion	0.40	81	87	2
Heptachlor epoxide	0.10	89	90	2
trans-Chlordane	0.05	100	93	1
cis-Permethrin	0.40	98	90	2
Parathion methyl	0.30	85	95	2
trans-Permethrin	0.40	99	85	2
Polychlorinated biphenyls (Aroclor 1254)	1.00	101	95	1
Endrin	0.10	10	18	2
Dicloran	0.10	84	87	2&3
Endosulfan I	0.10	93	83	2
p,p-TDE	0.10	95	98	1
Endosulfan II	0.10	64	63	2
Chlorpyrifos methyl	0.10	82	83	2
Fonofos	0.30	91	89	2&3
Chlorothal (DCPA)	0.10	97	97	2
Quintozene	0.05	91	86	1
Ethion	0.30	80	85	2
Tecnazene	0.05	89	93	1
Dimethoate[e]	0.40	0	0	NR
Phorate sulfone[e]	0.30	0	0	3
Demeton-*S*-sulfone[e]	0.40	0	0	NR
Malathion	0.40	85	80	3
Chlorpyrifos	0.15	85	83	2
cis-Chlordane	0.05	102	103	1
Octachlor epoxide	0.05	100	91	1
Endosulfan sulfate	0.15	100	94	2
Pentachlorobenzene	0.05	90	91	1
Pentachloroanisole	0.05	84	75	1
Phosalone	0.80	77	74	2&3
Methamidophos[e]	0.30	0	0	NR
Acephate[e]	0.40	0	0	NR
Omethoate[e]	0.40	0	0	NR
Tributyl PO_4[e]	0.40	0	0	3
Methidathion	0.40	75	81	3

Table 3. Continued

	Standard (μg)	Recovery (%) from 0–70 ml eluate		Alternate Florisil eluate[d]
		Column B	Column A	
EPN	0.60	80	84	2
Phosmet[e]	0.80	0	0	3
Tris (2-chloro-Et) PO_4[e]	0.40	0	0	NR
Trioctyl PO_4[e]	0.80	0	0	3
Tributoxy PO_4[e]	0.80	0	0	3
Octyl diphenyl PO_4[e]	0.60	0	0	3
Triphenyl PO_4[e]	0.60	0	0	3
Azinphos methyl[e]	1.00	0	0	NR
α-Mevinphos[e]	0.40	0	0	NR
β-Mevinphos[e]	0.40	0	0	NR
Tribufos (DEF)[e]	0.30	0	0	3
Chlordimeform[e]	0.30	0	0	NR
Atrazine[e]	0.30	0	0	3&D[a]
Cyanazine[e]	0.30	0	0	D
Anilazine[e]	0.80	0	0	2&3
Mirex	0.10	92	94	1
Dichlobenil	0.06	73	[f]	2
Chloroneb	0.06	83	75	2
Dicloran metabolite	0.10	98	87	3
Chlorothalonil	0.10	96	86	2&3
Chlorpropham metabolite	0.20	85	90	3
o,p-Dicofol	0.20	89	85	2
Chlorobenzilate	0.20	106	103	2&D
Methoxychlor olefin	0.20	86	80	2
Diclofop methyl	0.30	96	89	2
o,p-Methoxychlor	0.20	80	83	2
Fenarimol[e]	0.60	0	0	D
Propachlor[e]	0.20	0	0	1,2&3
Propanil[e]	0.20	0	0	3
Metolachlor[e]	0.20	0	0	1,2&3
Deltamethrin	2.00	75	83	2
Cypermethrin	1.00	90	90	2
Fluvalinate	0.80	90	90	2
Fenvalerate	2.00	75	92	2
2,3,5,6-Tetrachloronitroanisole	0.06	106	100	1
2,3,5,6-Tetrachloroaniline	0.06	102	87	1
2,3,5,6-Tetrachloroanisole	0.04	98	81	1
2,3,5,6-Tetrachlorothioanisole	0.04	78	78	1
2,3,5,6-Tetrachloroanisidine	0.04	96	92	1
1,2,3,5-Tetrachlorobenzene	0.02	83	92	1
1,2,4,5-Tetrachlorobenzene	0.02	92	85	1
Heptachloronorbornene	0.02	91	82	1
Hexachloronorbornadiene	0.08	64	66	1
Hexachlorocyclopentadiene	0.06	74	73	1
Vinclozolin	0.10	89	63	2
Captan[e]	0.20	0	0	3
Endrin aldehyde	0.10	136	171	15[b]&D
Endrin ketone	0.10	26	26	2
β-BHC	0.06	87	98	1
δ-BHC	0.06	85	92	1

Table 3. Continued

	Standard (μg)	Recovery (%) from 0–70 ml eluate		Alternate Florisil eluate[d]
		Column B	Column A	
o,p-DDE	0.10	92	98	1
Folpet[e]	0.20	0	0	1
o,p-TDE	0.10	107	110	1
o,p-DDT	0.10	88	97	1
Toxaphene	1.00	99	101	1
Chlordane, technical	1.00	80	82	1
Aldrin	0.40	94	93	1
Fenitrothion	0.40	87	83	2
Linuron[e]	2.00	0	0	3&D
Iprodione[e]	0.40	0	0	3&D
Iprodione isomer[e]	0.40	0	0	3&D
Iprodione metabolite[e]	0.40	0	0	D
Procymidone	0.10	88	103	15
Fenpropathrin	4.00	94	94	2
Flucythrinate	4.00	97	73	2
Tralomethrin	2.00	21	25	2
Pentachlorophenyl methyl sulfide (PCTA)	0.05	80	83	1
Chlorpropham	0.20	82	96	2
Bifenthrin	0.40	103	87	2
λ-Cyhalothrin	0.80	97	83	2
Cyfluthrin	0.80	95	95	2

[a] Elution mixture D is diethyl ether–light petroleum (1:1) and is the elution mixture of choice or completes the elution of the compound from the Florisil column.

[b] Elution mixture 15 is diethyl ether–light petroleum (15:85) and is the elution mixture of choice.

[c] ''&'' indicates that the elution of the compound splits between the different Florisil eluates.

[d] The alternate Florisil [2] elution mixtures or eluates are 1, 2 and 3.

[e] Compounds were not recovered through the automated C_1 system.

[f] Interference.

NR: indicates no recovery from Florisil.

Table 4
Amount of lipids contained in the pesticide fraction after being cleaned up through automated C_1 system[a]

Lipids	Lipid (g)	Lipids (mg) in 0–70 ml eluate	
		Column B	Column A
Butter	0.65	2.9	2.8
Corn oil	0.76	0.9	0.6
Soybean oil	0.76	1.1	1.2
Olive oil	0.76	2.7	2.6
Canola oil	0.76	1.4	1.4

[a] Gel permeation clean-up of 0.75 g of corn oil and 0.65 g butter leaves 2.4 mg and 3.2 mg, respectively of residue in the pesticide fraction.

fraction for each sample was less than 3 mg. The samples had fat ranging from 5–17.8% and moisture varying from 29–63%. The 3 mg of residue left in the sample pesticide fraction is comparable to the GPC clean-up used in the initial analysis.

Samples of pancakes and biscuits were analyzed six times for incurred pesticide residues using the automated C_1 system, and the results from both columns A and B are shown in Table 6. These results show that the clean-up system is reproducible and comparable to the traditional Total Diet Study results.

Fatty food samples in the Total Diet Study are analyzed for pesticide residues using the PAM I, section 304, E1, C5 and C6 methodology. The fat is extracted with mixed ethers and the pesticide residues are separated from the fat with gel permeation chromatography and the eluates are further cleaned

Table 5
Results (ppm=μg/g) for incurred pesticide residues in total diet food items (<18% fat and 70% moisture)

	TDS (n=1)	Automated C_1 system (n=1)	
		Column B	Column A
French fries			
(5% fat, 63% moisture)			
Chlorpyrifos methyl	0.001	0.0013	0.0011
Endosulfan sulfate	0.002	0.0016	0.0016
Chlorpropham (CIPC)	0.560	0.854	0.905
Chlorpropham metabolite	0.016	0.018	0.019
p,p′-DDE	0.001	0.001	0.001
p,p′-TDE	0.0004	0.0006	0.0004
p,p′-DDT	0.0005	0.0007	0.0006
Sample mass (g)	50	5	5
Hydromatrix (g)	NA	2.2	2.2
Residue (mg) in eluates	ND	1=1.1	1=0.8
(after Florisil)		2=0.7	2=0.8
		D=0.6	D=0.9
Fish sandwich on bun			
(13.4% fat, 48% moisture)			
Chlorpropham (CIPC)	0.002	0.0013	0.0016
Chlorpyrifos methyl	0.001	0.0012	0.0013
Dieldrin	0.0003	0.0008	0.0008
p,p′-DDE	0.0007	0.0008	0.0008
p,p′-Methoxychlor	0.0005	0.0006	0.0006
Sample mass (g)	50	5	5
Hydromatrix (g)	NA	2.2	2.2
Residue (mg) in eluates	ND	1=0.7	1=1.4
(after Florisil)		2=0.4	2=0.7
		D=0.8	D=0.6
Egg, cheese, ham on muffin			
(7.9% fat, 54% moisture)			
Chlorpyrifos methyl	0.002	0.0013	0.0011
Malathion	0.002	0.0013	0.0015
p,p′-DDE	0.003	0.0024	0.0029
Sample mass (g)	50	5	5
Hydromatrix (g)	NA	2.4	2.4
Residue (mg) in eluates	ND	1=0.7	1=1.4
(after Florisil)		2+D=0.5	2+D=1.0
Chocolate cake			
(12% fat, 29% moisture)			
Chlorpyrifos methyl	0.0008	0.0009	0.0005
Malathion	0.003	0.0042	0.0034
Sample mass (g)	50	5	5
Hydromatrix (g)	NA	2.7	2.7
Residue (mg) in eluates	ND	2.0	2.1
(no Florisil)			

Table 5. Continued

	TDS (n=1)	Automated C_1 system (n=1)	
		Column B	Column A
Lamb chops			
(17.8% fat, 49% moisture)			
Chlorpyrifos methyl	0.0002	0.0002	0.0002
p,p′-DDE	0.005	0.0058	0.0057
Sample mass (g)	50	5	5
Hydromatrix (g)	NA	2.2	2.2
Residue (mg) in eluates (no Florisil)	ND	0.9	1.2
Fish sticks			
(17% fat, 47% moisture)			
Chlorpyrifos methyl	0.001	0.0014	0.0015
Malathion	0.002	0.0027	0.0037
Sample mass (g)	50	5.2	5.2
Hydromatrix (g)	NA	2.4	2.4
Residue (mg) in eluates (no Florisil)	ND	1.6	0.3

up with Florisil. The above information has shown that the automated C_1 clean-up procedure is equivalent to the current procedure in most cases, but is not equivalent in all cases because every pesticide that comes through the current methodology does not come through the automated C_1 clean-up system.

Table 6
Reproducibility results (ppm) for incurred pesticide residues in total diet food items (<18% fat and 70% moisture)

	TDS (n=1)	Automated C_1 system		
		(n=6) Range	(n=6) (average)	(n=6) RSD (%)
Pancakes from mix				
(9% fat, 49% moisture)				
Chlorpyrifos methyl	0.012	0.0080–0.0094	0.0088	5.7
Malathion	0.020	0.020–0.0218	0.0208	3.4
Sample mass (g)	50	5.02–5.08	NA	NA
Hydromatrix (g)	NA	2	NA	NA
Residue (mg) in eluates (no Florisil)	ND	0.4–0.9	NA	NA
Biscuit, baking powder[a]				
(9.3% fat, 3% moisture)				
Chlorpyrifos methyl	0.0009	0.0010–0.0014	0.0013	16.5
Pirimiphos methyl	0.003	0.0029–0.0033	0.003	5.8
Sample mass (g)	50	5.02–5.06	NA	NA
Hydromatrix (g)	NA	2	NA	NA
Residue (mg) in eluates (no Florisil)	ND	0.3–0.6	NA	NA

[a] 0.5 ml of water added to each extraction vessel.

4. Conclusion

This automated clean-up procedure should be considered as an alternative clean-up procedure for specific pesticide residues in fats and fatty samples because it simplifies the extraction and clean-up step and reduces solvent consumption and hazardous waste.

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